**Historic, archived document**

Do not assume content reflects current scientific knowledge, policies, or practices.

Issued October 18, 1909.

U. S. DEPARTMENT OF AGRICULTURE,
BUREAU OF ANIMAL INDUSTRY.—BULLETIN 117.
A. D. MELVIN, CHIEF OF BUREAU.

# LEUCOCYTES IN MILK:

## METHODS OF DETERMINATION AND THE EFFECT OF HEAT UPON THEIR NUMBER.

BY

H. C. CAMPBELL, V. M. D., M. D.,
*Expert in Milk Hygiene, Pathological Division.*

**(In cooperation with the Pennsylvania State Live-Stock Sanitary Board.)**

WASHINGTON:
GOVERNMENT PRINTING OFFICE.
1909.

## THE BUREAU OF ANIMAL INDUSTRY.

*Chief:* A. D. Melvin.
*Assistant Chief:* A. M. Farrington.
*Chief Clerk:* Charles C. Carroll.
*Biochemic Division:* M. Dorset, chief.
*Dairy Division:* B. H. Rawl, chief.
*Inspection Division:* Rice P. Steddom, chief; Morris Wooden, R. A. Ramsay, and Albert E. Behnke, associate chiefs.
*Pathological Division:* John R. Mohler, chief.
*Quarantine Division:* Richard W. Hickman, chief.
*Zoological Division:* B. H. Ransom, chief.
*Experiment Station:* E. C. Schroeder, superintendent.
*Animal Husbandman:* George M. Rommel.
*Editor:* James M. Pickens.

# LETTER OF TRANSMITTAL.

U. S. Department of Agriculture,
Bureau of Animal Industry,
*Washington, D. C., July 12, 1909.*

Sir: I have the honor to transmit the accompanying manuscript entitled "Leucocytes in Milk: Methods of Determination and the Effect of Heat upon their Number," by Dr. H. C. Campbell, expert in milk hygiene of the Pathological Division of this Bureau. The experimental work described was conducted in cooperation with the Pennsylvania State live-stock sanitary board.

The presence of leucocytes in milk has excited a great deal of interest among investigators for some time past, and extensive examinations have been made to determine the significance of these cellular elements. The experiments undertaken by Doctor Campbell have enabled him to arrive at some definite conclusions, which are a valuable addition to previous knowledge of the subject. I therefore recommend the publication of the paper in the bulletin series of this Bureau.

The author asks that acknowledgment be made of many valuable suggestions by Dr. John R. Mohler, chief of the Pathological Division of the Bureau of Animal Industry; Dr. Leonard Pearson, State veterinarian of Pennsylvania; Dr. D. H. Bergey, of the laboratory of hygiene of the University of Pennsylvania; and Dr. John Reichel, of the laboratory of the Pennsylvania State live-stock sanitary board.

Very respectfully,

A. D. Melvin,
*Chief of Bureau.*

Hon. James Wilson,
*Secretary of Agriculture.*

# CONTENTS.

Page.
The composition and characteristics of cow's milk .......... 7
The presence of leucocytes in milk .......... 7
Various methods for determining the number of leucocytes in milk .......... 9
    A comparison of the methods .......... 11
Determination of leucocytes in milk after heating .......... 13
    In milk drawn directly from cow .......... 14
    In single samples of market milk .......... 15
    In duplicate samples of milk .......... 16
    Number in milk heated to varying temperatures .......... 17
    Number in milk after pasteurizing and cooling .......... 18
    Determinations by various methods .......... 19
Conclusions .......... 19

# LEUCOCYTES IN MILK: METHODS OF DETERMINATION AND THE EFFECT OF HEAT UPON THEIR NUMBER.

## THE COMPOSITION AND CHARACTERISTICS OF COW'S MILK.

Milk contains all the food elements necessary for the development of the young. Not only do these food elements vary as to their percentages in the milk of individuals belonging to any one species, but the variations are still greater between different species. At first thought it may seem easy to give a statement concerning the composition of average cow's milk, but if one studies the subject it will be found that many factors come into play which render it very difficult to determine just what average milk is.

The average chemical composition of cow's milk may be stated to be about as follows: Water, 87 per cent; fat, 3.9 per cent; casein, 2.7 per cent; albumen, 0.7 per cent; sugar, 5 per cent. Milk also contains cellular elements, the number in normal milk usually not exceeding 500,000 per cubic centimeter.

Just as the chemical constituents have been found to vary according to the period of lactation, health, and breed of the animal, so has the number of cellular elements been found to vary. There is no definite standard of normal milk as to its chemical, physical, and physiological characteristics. In consequence of this the exact line of demarcation between the physiologic and pathologic properties of milk is an arbitrary one and determinable only by careful study.

At the beginning of the first week of lactation cow's milk contains two kinds of cells—colostrum corpuscles and leucocytes. The former are recognized under the microscope by their size and granulations; the latter correspond in morphology to the polymorphonuclear cells of human blood, and are always present in small numbers in normal milk. A third kind of cell, the epithelial cell, frequently gains entrance to the milk in the course of milking, being derived from the teats of the cow or from the hands of the milker.

## THE PRESENCE OF LEUCOCYTES IN MILK.

It has long been known that milk contains many bacteria and cellular elements. The relation existing between the kind of bacteria and the cellular elements has been a subject of considerable discussion in the last few years. Early observers found that the leucocytes and bacteria were invariably increased in the milk from a cow with udder inflammation. When milk contained a large number of bacteria and cells, it was thought that some one animal in a herd was suffering from garget or mammitis. Later observers contended that these cellular elements were found in normal milk, and that different animals and even different quarters of the udder in the same animal contained varying amounts of these elements.

The variety of bacteria commonly associated with an increase of leucocytes is the streptococcus. Whether the polymorphonuclear leucocyte is to be considered as a pus cell or a normal leucocyte is still an unsettled question. The early milk experts were inclined to consider the presence of large numbers of leucocytes as an indication of udder disease, the reason for this being that they found increased numbers of the streptococci also present. Some later observers—for instance, Savage—did not find this relation. Since normal milk contains leucocytes in varying numbers, it is the writer's opinion that they can not be considered as pus cells unless they are present in large numbers, associated with pus-producing organisms. The mere presence of leucocytes in milk, therefore, does not seem to indicate pus formation in the udder.

Of the cellular elements found in milk the presence of leucocytes has excited the greatest interest among investigators, especially since Stokes conceived that the centrifugalized sediment containing more than 10 leucocytes to a field of a $\frac{1}{12}$ oil-immersion lens indicates the presence of pus in the milk and consequently inflammatory processes of the udder. It is only natural that considerable significance was given to this finding, and since then various investigators have undertaken extensive examinations in order to determine the significance of these leucocytes in milk. The results of the findings of recent observers indicate that the presence of large numbers of leucocytes in milk is indicative of an inflammation of the udder, viz, streptococcic mastitis. Exceptions of course will be found where large numbers of leucocytes appear during the first portion of the lactation period and, as some observers contend, when milking is carried on until late in the period of gestation.

The demonstration of a mastitis by the presence of leucocytes would be of very great importance, as it is a well-known fact that such an affection can not always be established by a clinical examination of the udder, especially in its early stages, and there are even instances when the affection might pass off without disclosing any clinical evidence of its existence. However, with the technique heretofore employed in the numerical determination of leucocytes, there is too narrow a margin between the leucocytes found in the milk of healthy cows and in that of diseased cows to make the majority of these methods of diagnosis satisfactory and practicable.

## VARIOUS METHODS FOR DETERMINING THE NUMBER OF LEUCOCYTES IN MILK.

Different observers have advised various methods for estimating the number of leucocytes in milk in order to establish a standard by which it may be judged. While the several methods recommended are not directly comparable, it is nevertheless evident that an entire lack of harmony exists at present among them, and that the reliability of one or more of the methods is at least doubtful.

The first investigator to adopt a standard was Stokes, of the Maryland State board of health. The next was Stewart, of the city board of health of Philadelphia. Later Doane and Buckley, of the Maryland Agricultural Experiment Station, devised what is at present considered by many to be the most accurate and uniform method. A similar method was proposed independently by Savage. Many others have done work along this line, but all have used modifications of the methods mentioned.

Three general methods are now in vogue, namely, (1) smearing the sediment on a glass slide; (2) a volumetric method; and (3) a determination of the percentage of sediment in the milk. The first method in a general way involves the principles suggested by Stokes and Stewart; the second is that originated by Doane and Buckley and by Savage; and the third method is that devised by Trommsdorff.

All of these methods for determining the number of leucocytes in milk have been used throughout this work, that of Doane and Buckley being slightly modified in some details, as is explained later on. A brief description of the technique used in the methods mentioned follows:

*Stokes's method.*—This consists in placing 10 c. c. of milk in a glass tube, which is whirled in an ordinary centrifuge for ten minutes. The fat and supernatant liquid are then poured off, and one platinum loopful of the sediment is spread over an area of 1 square centimeter on a glass cover slip. This is stained with methylene blue for one minute, and mounted in either water or Canada balsam on a glass slide. Ten fields are counted with a $\frac{1}{12}$ oil-immersion lens, and the average count is taken as the number per field. It has been the custom of those using this method to regard the milk as unfit for use when the number of leucocytes exceeds 10 per field.

*Stewart's method.*—This method consists in placing 1 c. c. of milk in a small glass tube, stoppered at one end with a rubber plug. The tube is placed on a Stewart disk, with the stoppered end at the edge of the disk, and is centrifuged for ten minutes. The rubber plug is drawn out while the tube is held horizontally, and the sediment on the rubber plug is smeared over an area of 1 square centimeter on a glass cover slip. This is stained for one minute with methylene blue and is mounted in either water or Canada balsam on a glass slide. Ten fields are counted with the $\frac{1}{12}$ oil-immersion lens and the average count is taken as the number per field. Those using this method are accustomed to regard the milk as unfit for use when the count exceeds 23 per field.

*Trommsdorff's method.*—This requires the use of a specially constructed tube holding 10 c. c. and drawn out at the bottom into a fine caliber. The narrow part of the tube has twenty graduations, each one representing 0.01 per cent of the sediment from 10 c. c. of milk. Ten cubic centimeters of milk are placed in the tube, which is whirled in an ordinary centrifuge for ten minutes. At the expiration of this time the tube is taken out and the percentage of sediment read directly from the tube. This method gives the percentage of sediment, but gives no information as to the substances of which it consists. Trommsdorff's method, however, appears to give satisfactory results in practice and is supported by many investigators as a relatively reliable method for practical use in demonstrating pus in milk.

*The Doane-Buckley method.*—This method consists in placing 10 c. c. of milk in a glass tube graduated in cubic centimeters. The tube is centrifuged for ten minutes, and then the fat and supernatant liquid are aspirated off down to the 1 c. c. mark. This remaining cubic centimeter is thoroughly mixed with a saturated alcoholic solution of methylene blue and is stained for one minute. One drop of this mixture is placed in a Thoma-Zeiss blood-counting apparatus and the number of leucocytes per cubic centimeter is determined. Those using this method consider the milk below standard when the number of cells exceeds 500,000 per cubic centimeter.

In the experiments here recorded, the writer found it better to aspirate off the fat and supernatant liquid down to the 5 or 6 c. c. mark, being careful not to allow the pipette to go below the surface of the liquid. The tube was then filled up to the 10 c. c. mark with distilled water and recentrifuged. This was repeated several times until the upper surface of the liquid was transparent, then the supernatant liquid was aspirated off down to the 1 c. c. mark. The 1 cubic centimeter of sediment remaining was thoroughly mixed, 1 drop of the sediment was placed in a blood-counting apparatus, and the number of leucocytes per cubic centimeter was estimated. The advantage of washing several times and of using no stain was that there were very few fat globules and no precipitate from the stain. With the other method these were frequently found to interfere with the making of an accurate count.

## A COMPARISON OF THE METHODS.

It seemed desirable that a comparison of the foregoing methods should be made. This was done, using the technique above described. In each case there was made a determination of the number of bacteria per cubic centimeter, and the various species of bacteria present were identified. Milk from three different sources was examined, namely, (1) market milk; (2) milk from a cow in her first lactation period (the cow had been under observation for some time and was known to have no udder lesions); (3) milk from a cow with inflammation of the udder. The results are set forth below in tabular form. Table 1 gives the results of examinations that were made of market milk.

TABLE 1.—*Determination of leucocytes and bacteria in market milk by various methods.*

| No. of sample. | Number of leucocytes. | | | Sediment (Trommsdorff). | Number of bacteria. | Streptococci. | Kinds of bacteria found. |
|---|---|---|---|---|---|---|---|
| | Stokes. | Stewart. | Doane-Buckley. | | | | |
| | *Per field.* | *Per field.* | *Per c. c.* | *Per cent.* | *Per c. c.* | | |
| 1...... | 5.0 | 10.0 | 34,000 | 0.05 | 9,080 | Present. | *Staphylococcus albus*, *Bacillus mesentericus.* |
| 2...... | 4.0 | 5.0 | 32,000 | .02 | 877 | ...do.... | *Staph. albus*, *Staph. aureus.* |
| 3...... | 2.0 | 2.0 | 48,000 | .01 | 413 | ...do.... | *Staph. albus.* |
| 4...... | 10.0 | 5.0 | 64,000 | .04 | 480 | ...do.... | *Sarcina alba.* |
| 5...... | 2.0 | 2.0 | 32,000 | .02 | 21,000 | ...do.... | B. Class IV (Chester, p. 155). |
| 6...... | 2.0 | 2.0 | 32,000 | .02 | 7,500 | ...do.... | *Staph. albus*, *Sarcina pulmonum.* |
| 7...... | 2.0 | 2.0 | 120,000 | .02 | 26,000 | ...do.... | *B. mesentericus*, *Mycobact. lactis.* |
| 8...... | 3.0 | 2.0 | 64,000 | .02 | 116,000 | ...do.... | *Bact. luteum.* |
| 9...... | 3.0 | 2.0 | 65,000 | .02 | 76,000 | ...do.... | *Staph. aureus.* |
| 10...... | 1.0 | 2.0 | 25,000 | .02 | 4,596,000 | ...do.... | *Staph. albus.* |
| 11...... | 1.0 | 2.0 | 28,000 | .01 | 304,000 | ...do.... | *Bact. lactis*, *Bact. limbatum.* |
| 12...... | 1.0 | 2.0 | 30,000 | .03 | 3,200,000 | ...do.... | |
| 13...... | 2.0 | 2.0 | 34,000 | .02 | 551,000 | ...do.... | *Mycobact. lactis.* |
| 14...... | 1.0 | 1.0 | 46,000 | .02 | 64,000 | ...do.... | |
| 15...... | .4 | .8 | 7,000 | .02 | 145,000 | ...do.... | *Staph. albus*, *micrococcus luteus.* |
| 16...... | 1.0 | 1.0 | 34,000 | .07 | 490,000 | ...do.... | |
| 17...... | 1.0 | 3.0 | 45,000 | .03 | 260,000 | ...do.... | *Bact. rugosum*, *Bact. plicatum*, *Mic. albus*, *B. sub flavus.* |
| 18...... | .5 | 1.0 | 15,000 | .02 | 30,000 | ...do.... | *B. circulans.* |
| 19...... | 2.0 | 2.0 | 38,000 | .03 | 200,000 | ...do.... | *B. liqefaciens*, *B. pseudotyphosus.* |
| 20...... | 5.0 | 7.0 | 125,000 | .05 | 150,000 | ...do.... | *B. mesentericus vulgatus*, *Mic. acidi lactici*, *Bact. acidi lacti.* |

These samples of market milk were bought from ordinary milk stores in Philadelphia. The various methods used in determining the leucocytes all showed a certain, although not a uniform, relationship; that is, when one ran high the others usually also showed a high count. Streptococci were found either on staining the sediment or on the agar plates in each of the 20 samples examined.

Table 2 shows the results obtained from the examination of the milk from a young cow in her first lactation period. The cow had been under observation for some time and was known to have no udder lesions.

TABLE 2.—*Determination of leucocytes and bacteria in milk from cow in first lactation period.*

| No. of sample. | Number of leucocytes. | | | Sediment (Trommsdorff). | Number of bacteria. | Kinds of bacteria found. |
|---|---|---|---|---|---|---|
| | Stokes. | Stewart. | Doane-Buckley. | | | |
| | *Per field.* | *Per field.* | *Per c.c.* | *Per cent.* | *Per c.c.* | |
| 1...... | 8.0 | 3.0 | 61,000 | 0.03 | 1,833 | *Micrococcus orbiculatus, Mycobact. pseudo-diphtheria.* |
| 2...... | 1.0 | 1.0 | 19,000 | .02 | 8,700 | *Mic. orbiculatus, Mic. ovalis.* |
| 3...... | 1.0 | 1.0 | 19,000 | .02 | 4,122 | *Mic. orbiculatus, Mycobact. pseudo-diphtheria.* |
| 4...... | .1 | 1.0 | 7,000 | .01 | 3,700 | *Bacillus liquefaciens, Mic. acidi lactici.* |
| 5...... | 1.0 | 5.0 | 8,000 | .01 | 2,833 | *Mic. acidi lactici.* |
| 6...... | .8 | 4.0 | 9,000 | .01 | 5,550 | *Mycobact. pseudo-diphtheria.* |
| 7...... | .5 | 3.0 | 2,000 | .0 | 1,000 | *Mic. luteus, Mic. acidi lactici.* |
| 8...... | .5 | 1.0 | 4,000 | .0 | 1,466 | *Mic. ovalis, Mycobact. lactis.* |
| 9...... | .5 | 2.0 | 5,000 | .0 | 2,061 | *Mycobact. lactis.* |
| 10...... | 1.0 | 7.0 | 3,000 | .01 | 1,505 | *Mic. acidi lactici.* |
| 11...... | 1.5 | 7.0 | 10,000 | .015 | 1,600 | *Mycobact. lactis.* |
| 12...... | .3 | 5.0 | 8,000 | .01 | 733 | *Bact. mycoides, Mic. lactis.* |
| 13...... | .7 | 1.0 | 10,000 | .01 | 811 | *Mycobact. lactis.* |
| 14...... | .6 | 1.0 | 12,000 | .01 | 8,300 | *Mic. luteus, Sarcina alba, Mic. acidi lactici.* |
| 15...... | .4 | 7.0 | 7,000 | .01 | 927 | B. Class II (Chester, p. 227), *Mic. acidi lactici.* |
| 16...... | 1.0 | 1.0 | 14 500 | .01 | 7,000 | *B. mesentericus, Sarcina lutea, Mic. acidi lactici.* |
| 17...... | 1.0 | 1.0 | 16,000 | .01 | 333 | *B. mesentericus, Sarcina lactis.* |
| 18...... | 2.0 | 1.5 | 14,000 | .02 | 10,000 | *B. mesentericus.* |
| 19...... | 4.0 | 4.0 | 81,000 | .02 | 672 | *B. mesentericus, Mic. aerius, Mycobact. lactis.* |
| 20...... | 4.0 | 3.0 | 20,000 | .02 | 16,000 | *B. mesentericus, Mic. acidi lactici, Sarcina lactis.* |

The above samples of milk were taken in a careful manner from a cow at the experiment farm of the Pennsylvania State live-stock sanitary board, in Delaware County. The cow's udder and the milker's hands were washed with a 1 per cent solution of creolin and wiped dry with sterile absorbent cotton. The milk was drawn directly from the udder into a sterile glass bottle, which was then plugged with sterile cotton and brought at once to the laboratory for examination. One sample was taken on every other day, excluding Sundays. In all of the samples the leucocytic count and the percentage of sediment were very low, considered with relation to the standard. No streptococci were found either in an examination of the sediment or on the agar plates.

Table 3 shows milk examined in the same manner from a cow having inflammation of the udder.

TABLE 3.—*Determination of leucocytes and bacteria in milk from cow having inflammation of the udder.*

| No. of sample. | Number of leucocytes. | | | Sediment (Trommsdorff). | Number of bacteria. | Kinds of bacteria found. |
|---|---|---|---|---|---|---|
| | Stokes. | Stewart. | Doane-Buckley. | | | |
| | *Per field.* | *Per field.* | *Per c. c.* | *Per cent.* | *Per c. c.* | |
| 1...... | 17 | 20 | 425,000 | 0.03 | 5,450 | *Bacillus mesentericus, B. citreus, Mycobact. pseudo-diphtheria, Mycobact. lactis.* |
| 2...... | 3 | 5 | 80,000 | .01 | 300,000 | *B. mesentericus, Mycobact. pseudo-diphtheria.* |
| 3...... | 4 | 12 | 112,000 | .01 | 9,666 | B. Class II (Chester, p. 227), *Strep. mirabilis, B. rubus.* |
| 4...... | 1 | 5 | 14,000 | .005 | 350 | *B. aerogenes, Mic. citreus, B. rubus.* |
| 5...... | 4 | 4 | 25,000 | .005 | 1,000 | *B. mesentericus*, B. Class II (Chester, p. 227). |
| 6...... | 6 | 8 | 95,000 | .005 | 500 | *B. aerogenes, Mic. citreus, B. rubus.* |
| 7...... | 10 | 36 | 650,000 | .03 | 8,200 | *B. mesentericus, B. fluggei, Mycobact. lactis.* |
| 8...... | 50 | 60 | 720,000 | .04 | 1,200 | *Sarcina lutea, Mycobact. lactis.* |
| 9...... | 11 | 18 | 256,000 | .01 | 6,300 | *Sarcina lutea, Sarcina alba.* |
| 10...... | 13 | 19 | 142,000 | .015 | 250,000 | *Mic.acidi lactici,Sarcina lutea,B.columbarum.* |
| 11...... | 7 | 17 | 120,000 | .015 | 9,000 | Streptococci. |
| 12...... | 6 | 11 | 100,000 | .01 | 2,000 | *Mycobact. lactis, Sarcina lactis.* |
| 13...... | 13 | 16 | 136,000 | .015 | 600 | *B. javaniensis*, streptococci. |
| 14...... | 16 | 17 | 146,000 | .015 | 9,000 | Streptococci. |
| 15...... | 13 | 24 | 138,000 | .02 | 800 | *B. rubus*, streptococci. |
| 16...... | 3 | 4 | 100,000 | .01 | 5,200 | *B. rubefaciens, Bact. acidi lacti*, streptococci. |
| 17...... | 6 | 4 | 78,000 | .01 | 800 | *B. rubus, Sarcina lutea*, streptococci. |
| 18...... | 2 | 4 | 108,000 | .01 | 166,400 | Streptococci. |
| 19...... | 17 | 17 | 89,000 | .01 | 3,300 | Streptococci. |
| 20...... | 11 | 11 | 78,000 | .01 | 2,000 | Saccharomyces streptococci. |

The above samples were taken from a cow on the same farm and under the same precautions and in the same manner as observed in gathering the milk reported on in Table 2.

This cow gave birth to a calf on May 23, 1908. Immediately following this one quarter of the udder became inflamed and the secretion of milk from this quarter ceased. On June 25, when the first sample was taken for examination, this quarter had become atrophied and very hard. The samples were a mixture from the other three quarters. The majority of them showed an extremely large number of leucocytes. In 50 per cent of the examinations streptococci were found either from the sediment or on the agar plates. There seems to be no doubt that the cow's udder was infected with streptococci. The table seems to show that there is some relation between the presence of streptococci and the number of leucocytes. The writer has frequently observed that the streptococci which occur with large numbers of leucocytes are in narrow curved chains, and it appears that pathogenic streptococci are usually of this type. On the other hand, some forms of lactic-acid bacteria may be mistaken for small, short, and thick chains of streptococci. Therefore it appears that narrow, curved chains of streptococci occurring coincidently with large numbers of leucocytes should be regarded seriously and as evidence that the milk is unfit for consumption.

## DETERMINATION OF LEUCOCYTES IN MILK AFTER HEATING.

During the progress of the work previously described, an article by H. L. Russell and Conrad Hoffman upon "The Effect of Heating upon the Determination of the Leucocytes in Milk" appeared in the

August, 1908, number of the American Journal of Public Hygiene. This paper called attention to the apparent increase in the number of leucocytes in milk following heating. It appeared to be desirable to repeat their work under different conditions. Accordingly the following experiments were made, the details of which are shown in Tables 4 to 9. The Doane-Buckley method was used by Russell and Hoffman, whereas in our work the modification before described was employed.

### IN MILK DRAWN DIRECTLY FROM COW.

The first series of comparisons between heated and unheated milk were carried out with milk freshly drawn from the cows. In each instance the fore milk, middle milk, and strippings were studied. The sample was first examined raw; then it was heated to 70° C. and another count of leucocytes was made. The percentage of increase or decrease in the heated sample is shown in the last column of the following table, which gives the results in detail.

TABLE 4.—*Leucocyte content of heated and unheated milk freshly drawn from cow, showing variation between foremilk, middle milk, and strippings.*

| No. of cow. | Parts of same milking. | Cellular content of milk— | | Percentage of increase or decrease. |
|---|---|---|---|---|
| | | Unheated. | Heated to 70° C. | |
| | | *Per c. c.* | *Per c. c.* | |
| 800 | Foremilk | 316,000 | 2,800,000 | + 786 |
| | Middle milk | 440,000 | 4,000,000 | + 809 |
| | Strippings | 572,000 | 6,240,000 | + 990 |
| 800 | Foremilk | 1,800,000 | 11,500,000 | + 538 |
| | Middle milk | 1,600,000 | 7,200,000 | + 350 |
| | Strippings | 2,800,000 | 9,600,000 | + 242 |
| 708 | Foremilk | 16,000 | 45,000 | + 181 |
| | Middle milk | 37,000 | 112,000 | + 202 |
| | Strippings | 136,000 | 236,000 | + 73 |
| 800 | Foremilk | 4,400,000 | 6,800,000 | + 54 |
| | Middle milk | 2,000,000 | 3,200,000 | + 60 |
| | Strippings | 5,200,000 | 9,600,000 | + 84 |
| 708 | Foremilk | 80,000 | 1,200,000 | + 50 |
| | Middle milk | 380,000 | 488,000 | + 28 |
| | Strippings | 360,000 | 432,000 | + 20 |
| 800 | Foremilk | 20,480,000 | 8,400,000 | — 59 |
| | Middle milk | 1,260,000 | 2,798,000 | + 122 |
| | Strippings | 2,880,000 | 3,456,000 | + 20 |
| 722 | Foremilk | 128,000 | 240,000 | + 87 |
| | Middle milk | 192,000 | 392,000 | + 104 |
| | Strippings | 660,000 | 1,180,000 | + 78 |
| 806 | Foremilk | 66,000 | 25,000 | — 62 |
| | Middle milk | 30,000 | 42,000 | + 40 |
| | Strippings | 82,000 | 124,000 | + 51 |
| 720 | Foremilk | 92,000 | 288,000 | + 213 |
| | Middle milk | 80,000 | 340,000 | + 325 |
| | Strippings | 200,000 | 1,400,000 | + 600 |
| 797 | Foremilk | 160,000 | 236,000 | + 47 |
| | Middle milk | 288,000 | 597,000 | + 107 |
| | Strippings | 488,000 | 1,158,000 | + 137 |
| 797 | Foremilk | 60,000 | 156,000 | + 160 |
| | Middle milk | 187,000 | 210,000 | + 12 |
| | Strippings | 192,000 | 344,000 | + 78 |
| 761 | Foremilk | 67,000 | 240,000 | + 258 |
| | Middle milk | 96,000 | 136,000 | + 41 |
| | Strippings | 221,000 | 312,000 | + 41 |
| 716 | Foremilk | 60,000 | 176,000 | + 193 |
| | Middle milk | 42,000 | 128,000 | + 204 |
| | Strippings | 92,000 | 320,000 | + 247 |
| 718 | Foremilk | 448,000 | 1,300,000 | + 190 |
| | Middle milk | 310,000 | 608,000 | + 95 |
| | Strippings | 460,000 | 6,716,000 | +1,360 |

In the 42 samples here reported, 40 show that a much larger number of cells was found in the heated samples than in the unheated, while only two showed a decrease. The figures seem to indicate that heating causes a large increase in the number of leucocytes found on examination. The reason for this condition Doctor Russell explains in the following manner:

> When milk is heated for ten minutes at 60° C. the creaming power is greatly diminished. The fat-globule clusters are broken down and the butterfat is more or less homogeneously distributed throughout the milk serum. These fat-globule aggregations rise steadily to the surface and probably entangle a good many of the cell elements; but with the more homogeneous emulsion of the fat due to the action of the heat the leucocytes are probably not enmeshed and are therefore free to respond to the action of gravity.

The writer concurs in this explanation, since from the very nature of the leucocyte cell there can be no multiplication of these cells and therefore no actual increase in the number of leucocytes. A larger number is found in heated than in unheated milk because they more freely settle with the sediment as a result of becoming disentangled from the fat globules, due to the effect of the heat. In unheated milk, on the other hand, only a comparatively small proportion of the leucocytes settle with the sediment.

### IN SINGLE SAMPLES OF MARKET MILK.

The next table shows a number of counts made from single samples of market milk. In each case a number of subsamples were examined, but only one count was made from a given tube. For each count throughout all the work shown in this table a new tube was taken, centrifuged, and examined.

TABLE 5.—*Leucocyte content of heated and unheated market milk.*

| No. of sample. | Number of leucocytes in milk— | | Percentage of increase. |
|---|---|---|---|
| | Unheated. | Heated to 70° C. | |
| | *Per c. c.* | *Per c. c.* | |
| 1 | 41,000 | 584,000 | 1,324 |
| | 102,000 | 592,000 | 480 |
| | 85,000 | 612,000 | 620 |
| | 64,000 | 632,000 | 887 |
| | 46,000 | 626,000 | 1,263 |
| | 70,000 | 600,000 | 757 |
| | 72,000 | 636,000 | 783 |
| | 42,000 | 608,000 | 1,347 |
| | 21,000 | 580,000 | 2,661 |
| | 96,000 | 608,000 | 533 |
| | 23,000 | 616,000 | 2,578 |
| | 58,000 | 604,000 | 941 |
| | 73,000 | 664,000 | 809 |
| | 60,000 | 648,000 | 980 |
| | 34,000 | 632,000 | 1,758 |
| 2 | 102,000 | 492,000 | 382 |
| | 74,000 | 500,000 | 575 |
| | 100,000 | 532,000 | 432 |
| | 90,000 | 512,000 | 468 |

TABLE 5.—*Leucocyte content of heated and unheated market milk*—Continued.

| No. of sample. | Number of leucocytes in milk— | | Percentage of increase. |
|---|---|---|---|
| | Unheated. | Heated to 70° C. | |
| | *Per c. c.* | *Per c. c.* | |
| 2 | 65,000 | 498,000 | 666 |
| | 60,000 | 488,000 | 700 |
| | 54,000 | 516,000 | 855 |
| | 45,000 | 496,000 | 1,002 |
| | 40,000 | 524,000 | 1,210 |
| | 51,000 | 504,000 | 888 |
| | 64,000 | 496,000 | 675 |
| | 42,000 | 552,000 | 1,212 |
| | 56,000 | 506,000 | 803 |
| | 43,000 | 490,000 | 1,039 |
| | 38,000 | 510,000 | 1,241 |
| | 43,000 | 540,000 | 1,156 |
| 3 | 50,000 | 256,000 | 412 |
| | 62,000 | 264,000 | 325 |
| 4 | 70,000 | 1,600,000 | 2,185 |
| | 64,000 | 1,700,000 | 2,556 |
| | 78,000 | 1,600,000 | 1,938 |
| | 62,000 | 1,400,000 | 2,158 |
| 5 | 120,000 | 312,000 | 160 |
| | 106,000 | 308,000 | 190 |
| 6 | 96,000 | 272,000 | 183 |
| | 108,000 | 284,000 | 162 |
| | 112,000 | 288,000 | 162 |
| | 136,000 | 262,000 | 92 |
| 7 | 60,000 | 120,000 | 100 |
| | 32,000 | 118,000 | 268 |
| | 46,000 | 112,000 | 143 |
| | 58,000 | 118,000 | 103 |
| 8 | 162,000 | 222,000 | 37 |
| | 161,000 | 216,000 | 34 |
| | 156,000 | 196,000 | 25 |
| | 164,000 | 200,000 | 21 |

In this, as in Table 4, the heated sample in every case shows a marked increase in the number of leucocytes, and in many cases the increases are large.

## IN DUPLICATE SAMPLES OF MILK.

In the next table the results of the examination of a number of duplicate samples are shown. A study of this table shows that the analytical error is much smaller in samples heated to 70° C. than in unheated samples.

TABLE 6.—*Leucocyte content of duplicate samples of unheated and heated milk.*

| No. of sample. | Unheated. | | | Heated. | | |
|---|---|---|---|---|---|---|
| | First examination. | Second examination. | Variation. | First examination. | Second examination. | Variation. |
| | *Number.* | *Number.* | *Per cent.*[a] | *Number.* | *Number.* | *Per cent.*[a] |
| 1 | 41,000 | 102,000 | 59.9 | 548,000 | 592,000 | 7.4 |
| | 85,000 | 64,000 | 24.7 | 612,000 | 632,000 | 3.1 |
| | 46,000 | 70,000 | 34.2 | 626,000 | 600,000 | 4.1 |
| | 72,000 | 42,000 | 41.6 | 636,000 | 608,000 | 4.4 |
| | 21,000 | 96,000 | 78.1 | 580,000 | 608,000 | 4.6 |
| | 23,000 | 58,000 | 60.1 | 616,000 | 604,000 | 1.9 |
| | 73,000 | 60,000 | 17.8 | 664,000 | 648,000 | 2.4 |

[a] The smaller number is used as the basis in each case.

TABLE 6.—*Leucocyte content of duplicate samples of unheated and heated milk*—Cont'd.

| No. of sample. | Unheated. | | | Heated. | | |
|---|---|---|---|---|---|---|
| | First examination. | Second examination. | Variation. | First examination. | Second examination. | Variation. |
| | *Number.* | *Number.* | *Per cent.* | *Number.* | *Number.* | *Per cent.* |
| 2 | 74,000 | 100,000 | 26.0 | 500,000 | 532,000 | 6.0 |
| | 90,000 | 65,000 | 27.7 | 512,000 | 498,000 | 3.9 |
| | 54,000 | 45,000 | 16.6 | 516,000 | 496,000 | 4.0 |
| | 40,000 | 51,000 | 21.5 | 524,000 | 504,000 | 3.9 |
| | 64,000 | 42,000 | 34.3 | 496,000 | 552,000 | 11.3 |
| | 56,000 | 43,000 | 23.2 | 506,000 | 490,000 | 3.2 |
| | 38,000 | 43,000 | 17.6 | 510,000 | 540,000 | 5.8 |
| 3 | 50,000 | 62,000 | 19.3 | 256,000 | 264,000 | 3.0 |
| 4 | 70,000 | 64,000 | 8.5 | 1,600,000 | 1,700,000 | 5.8 |
| | 78,000 | 62,000 | 20.5 | 1,600,000 | 1,400,000 | 12.5 |
| 5 | 120,000 | 106,000 | 11.6 | 312,000 | 308,000 | 1.2 |
| 6 | 96,000 | 108,000 | 11.1 | 272,000 | 284,000 | 4.1 |
| | 112,000 | 136,000 | 17.6 | 288,000 | 262,000 | 9.0 |
| 7 | 60,000 | 32,000 | 46.6 | 120,000 | 118,000 | 1.6 |
| | 46,000 | 58,000 | 20.6 | 112,000 | 118,000 | 5.0 |
| 8 | 162,000 | 161,000 | .6 | 222,000 | 216,000 | 2.2 |
| | 156,000 | 164,000 | 4.8 | 196,000 | 200,000 | 2.0 |

Of the 24 duplicate samples examined, unheated and heated, the average variation in the unheated was 27.1 per cent as compared with only 4.2 per cent in the heated samples. These figures would seem to indicate that in routine inspection of milk one should heat the milk to somewhere near 70° C. before making an examination for leucocytes.

## NUMBER IN MILK HEATED TO VARYING TEMPERATURES.

After noting the above results, a number of samples were examined at different temperatures in order to observe at what point the change in the apparent leucocytic content occurred. The results are set forth in Table 7.

TABLE 7.—*Leucocyte content of milk at different temperatures.*

| No. of sample. | 15° C. | 30° C. | 40° C. | 50° C. | 60° C. | 70° C. | 80° C. | 90° C. | 100° C. for 5 minutes. |
|---|---|---|---|---|---|---|---|---|---|
| | *Per c. c.* | *Per c. c.* | *Per c. c.* | *Per c. c.* | *Per c. c.* | *Per c. c.* | *Per c. c.* | *Per c. c.* | *Per c. c.* |
| 1 | 70,000 | 80,000 | 140,000 | 158,000 | 1,200,000 | 1,600,000 | 1,600,000 | .......... | .......... |
| | 64,000 | 92,000 | 148,000 | 120,000 | 1,096,000 | 1,700,000 | 1,440,000 | .......... | .......... |
| | 78,000 | 84,000 | 144,000 | 154,000 | 1,120,000 | 1,600,000 | 1,640,000 | .......... | .......... |
| | 62,000 | 58,000 | 132,000 | 150,000 | 1,056,000 | 1,400,000 | 1,560,000 | .......... | .......... |
| 2 | 60,000 | 40,000 | 76,000 | 128,000 | 184,000 | 196,000 | 198,000 | 576,000 | 920,000 |
| | 48,000 | 32,000 | 100,000 | 132,000 | 176,000 | 194,000 | 200,000 | 592,000 | 960,000 |
| | 36,000 | 38,000 | 104,000 | 140,000 | 204,000 | 204,000 | 192,000 | 600,000 | 980,000 |
| | 40,000 | 46,000 | 108,000 | 164,000 | 188,000 | 208,000 | 196,000 | 650,000 | 1,000,000 |
| 3 | 144,000 | 136,000 | 132,000 | 140,000 | 184,000 | 256,000 | 704,000 | 724,000 | 960,000 |
| | 124,000 | 112,000 | 104,000 | 146,000 | 180,000 | 258,000 | 640,000 | 716,000 | 1,086,000 |
| | 106,000 | 124,000 | 128,000 | 144,000 | 186,000 | 260,000 | 716,000 | 680,000 | 946,000 |
| | 104,000 | 116,000 | 114,000 | 146,000 | 188,000 | 244,000 | 648,000 | 728,000 | 1,216,000 |
| 4 | 36,000 | 26,000 | 42,000 | 126,000 | 140,000 | 170,000 | 356,000 | 660,000 | 600,000 |
| | 38,000 | 36,000 | 56,000 | 130,000 | 148,000 | 154,000 | 308,000 | 580,000 | 610,000 |
| | 18,000 | 44,000 | 78,000 | 120,000 | 139,000 | 158,000 | 360,000 | 670,000 | 630,000 |
| | 42,000 | 28,000 | 60,000 | 124,000 | 145,000 | 144,000 | 380,000 | 590,000 | 650,000 |

The most satisfactory results were obtained between 60° C. and 70° C. There was an increase at 80, 90, and 100° C., but the results were not so uniform and the examination was much more difficult to make on account of the coagulated albumen, which interfered with the count. At the high temperatures the leucocytes were more or less disintegrated and could not be so easily differentiated from foreign material, hence the count was rendered less reliable.

### NUMBER IN MILK AFTER PASTEURIZING AND COOLING.

In pasteurized milk the leucocyte count is usually higher than in raw milk; therefore it was thought desirable to examine a series of four tubes from the same sample. The first tube was unheated; the second was heated to 70° C.; the third was heated and then cooled, and kept at 15° C. for twenty-four hours; the fourth was heated, then cooled, kept at 15° C. for twenty-four hours, and then heated again. The purpose of these experiments was to see if the milk after being pasteurized and cooled returned to the same condition as regards the number of leucocytes as that in which it was found before pasteurization.

Table 8 gives the results of these experiments, and indicates that there may be here a principle upon which may be based another method for determining whether milk has been heated or pasteurized.

TABLE 8.—*Leucocyte content of milk before and after heating and reheating.*

| No. of sample. | First examination. | | Second examination (after cooling at 15° C. for 24 hours). | |
|---|---|---|---|---|
| | Unheated. | Heated to 70° C. | Heated and cooled. | Heated second time to 70° C. |
| | *Per c. c.* | *Per c. c.* | *Per c. c.* | *Per c. c.* |
| 1 | 30,000 | 430,000 | 416,000 | 404,000 |
| | 19,000 | 428,000 | 424,000 | 412,000 |
| | 28,000 | 436,000 | 428,000 | 416,000 |
| | 26,000 | 432,000 | 360,000 | 422,000 |
| 2 | 260,000 | 380,000 | 452,000 | 348,000 |
| | 226,000 | 384,000 | 420,000 | 340,000 |
| | 208,000 | 380,000 | 440,000 | 346,000 |
| | 288,000 | 382,000 | 460,000 | 320,000 |
| 3 | 80,000 | 212,000 | 236,000 | 196,000 |
| | 60,000 | 204,000 | 228,000 | 204,000 |
| | 92,000 | 224,000 | 240,000 | 216,000 |
| | 76,000 | 222,000 | 208,000 | 200,000 |
| 4 | 60,000 | 248,000 | 214,000 | 268,000 |
| | 74,000 | 250,000 | 204,000 | 240,000 |
| | 78,000 | 240,000 | 216,000 | 264,000 |
| | 96,000 | 256,000 | 208,000 | 270,000 |

The table shows that in every instance after the milk was once heated the cooling did not bring it back to its original condition, while the second heating to 70° C. did not increase the count over the first heating.

### DETERMINATIONS BY VARIOUS METHODS.

The writer's modification of the Doane-Buckley method, which was considered the most accurate, was the only one used in the routine determinations of the number of leucocytes found in heated milk, as reported in Tables 4 to 8, inclusive. For the sake of comparison, therefore, a number of samples were examined in similar manner by the other methods, namely, those of Stokes, Stewart, and Trommsdorff. The results which are shown in Table 9 indicate that there are also more leucocytes found in heated milk than in unheated milk by each of these methods of determination.

TABLE 9.—*Leucocyte content of heated and unheated milk, as determined by Stokes, Stewart, and Trommsdorff methods.*

| No. of sample. | Milk unheated. | | | Milk heated to 70° C. | | |
|---|---|---|---|---|---|---|
| | Stokes. | Stewart. | Trommsdorff. | Stokes. | Stewart. | Trommsdorff. |
| | *No. per field.* | *No. per field.* | *Per cent sediment.* | *No. per field.* | *No. per field.* | *Per cent sediment.* |
| 1 | 0.6 | 1.8 | 0.005 | 5.1 | 13 | 0.03 |
| | .5 | 1.9 | .005 | 10.1 | 12 | .03 |
| | .2 | 2.7 | .005 | 8.0 | 7 | .03 |
| | .3 | 1.0 | .005 | 4.0 | 10 | .03 |
| 2 | 3.3 | 13.0 | .02 | 6.0 | 24 | .03 |
| | 3.5 | 14.0 | .02 | 15.0 | 22 | .03 |
| | 3.7 | 9.0 | .02 | 8.0 | 15 | .03 |
| | 1.8 | 10.0 | .02 | 12.0 | 12 | .03 |
| 3 | .3 | 1.5 | .01 | 3.0 | 6 | .02 |
| | .2 | .5 | .01 | 2.7 | 4 | .02 |
| | .1 | 1.6 | .01 | 4.3 | 8 | .02 |
| | 1.0 | 1.0 | .01 | 6.0 | 5 | .02 |
| 4 | .2 | .1 | .01 | .4 | 3 | .02 |
| | .1 | .15 | .01 | .5 | 2 | .02 |
| | .1 | .2 | .01 | .3 | 6 | .02 |
| | .15 | .1 | .01 | .6 | 4 | .02 |

## CONCLUSIONS.

1. The effect of heating upon the number of leucocytes found in milk increased the number of leucocytes per field in Doane and Buckley's, Stokes's, and Stewart's methods, also the percentage of sediment in the Trommsdorff method, in all of the samples examined.
2. It appears that heating is as essential as any other part of the technique in determining as nearly as possible the number of cellular elements in milk.
3. Since heating greatly increases the number in the count of the leucocytes, it seems necessary that a higher leucocyte standard shall be adopted in judging milk.
4. Milk inspection may be greatly benefited by the establishment of some rational standard for the leucocyte content of milk, but more study must be given to this subject in order to obtain the desired knowledge.

○